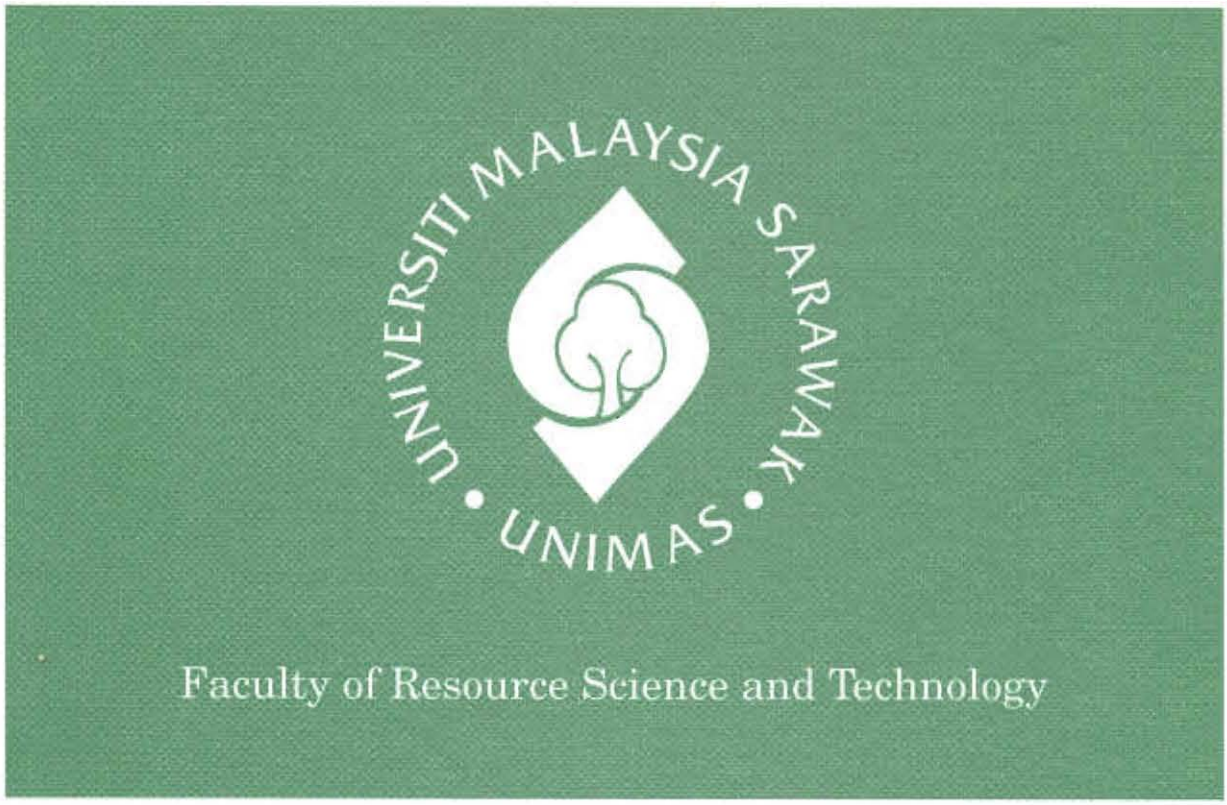


# COMPARISON OF DECAY SUSCEPTIBILITY AMONG TIMBER SPECIES AS SUITABLE CONTROLS IN WOOD DURABILITY TEST

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Bachelor of Science with Honours
(Plant Resource Scinece and Management)
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# COMPARISON OF DECAY SUSCEPTIBLITY AMONG TIMBER SPECIES AS SUITABLE CONTROLS IN WOOD DURABILITY TEST



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# Comparison of decay susceptibility among timber species as suitable controls in wood durability test

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## ABSTRACT

Duration of decay test was done between Rubberwood (*Hevea brasiliensis*), Ramin *(Gonystylus* sp*)*, Kempas (*Koompassia malaccensis*), Pelai *(Alstonia* sp) and Seladah (*family Burseacae*) by exposed to fungi *Chaetomium globosum* and *Schizophyllum commune*. The degree of fungi infection was recorded during three, six, nine and twelve weeks. The percentage mass loss and final moisture content were the parameter used in this study. Mass loss on wood was compared before and after fungi infection in order to determine which wood is more susceptible. Through the study, overall, Rubberwood showed the highest percentage mass loss (51.6%) infected by *Chaetomium globosum* and (35.9%) *Schizophyllum commune* while Kempas (1.2% & 1.6%), showed the lowest percentage of mass loss to either fungi infected. The percentage mass loss of Pelai (2.8% & 23.6%), Seladah (16.3% & 5.0%), and Ramin (3.1% & 3.3%) to the two fungi respectively were obtained. Obviously, Rubberwood was the most susceptible while Kempas was most durable against either *Chaetomium globosum* or *Schizophyllum commune* among the selected timber species.

Key words: Decay test, mass loss, *Chaetomium globosum*, *Schizophyllum commune*, light hardwood.

## *ABSTRAK*

*Ujian jangkitan kulat Chaetomium globosum dan Schizophyllum commune dijalankan keatas Rubberwood (Hevea brasiliensis) Ramin (Gonystylus* sp*), Kempas (Koompassia malaccensis), Pelai (Alstonia* sp*) dan Seladah (family Burseacae). Kadar serangan kedua kulat ini dicatatkan untuk tiga, enam, sembilan dan dua belas minggu. Dalam ujikaji ini, peratusan berat hilang dan peratusan kandungan air pada kayu adalah dua parameter penting yang digunakan. Walau bagaimanapun, berat kayu sebelum dan selepas dijangkiti kulat dibandingkan untuk menentukan kayu mana yang kurang resistant terhadap serangan kulat. Daripada kajian, secara keseluruhan Rubberwood mencatatkan peratusan berat hilang yang tinggi (51.6%) untuk jangkitan Chaetomium globosum dan (35.9%) untuk jangkitan Schizophyllum commune sementara kayu Kempas (1.2% & 1.6%) mencatatkan peratusan berat yang terendah pada salah satu jangkitan kulat. Peratusan berat yang hilang untuk Pelai (2.8% & 23.6%), Seladah( 16.3% & 5.0%), dan Ramin (3.1% & 3.3%) untuk jangkitan salah satu kulat. Jelaslah disini, Rubberwood adalah kayu yag mempunyai darjah ketahanan yang rendah terhadap kedua jangkitan kulat ini manakala kayu Kempas pula merupakan kayu yang lebih tahan terhadap jangkitan kulat Chaetomium globosum atau Schizophyllum commune berbanding kayu yang lain.*

*Kata kunci: Jangkitan kulat, kehilangan berat, Chaetomium globosum, Schizophyllum commune, light hardwood.*

# CHAPTER ONE

# INTRODUCTION

## 1.1 Wood Durability and Decay Fungi

According to Panshin & de Zeeuw (1980), wood durability can be defined as the capability of wood to resist the attack of wood destroying organisms including fungi, insects and marine borers. Comparing to the other plant tissue, wood is more resistant to deterioration from the attack of microorganism including fungi. This is because of the characteristic constituents in wood cell walls. The constituents present in wood cell walls such as extractives which sometimes bring the toxicity character that retard the growth of fungi. Natural durability of some species of wood is the presence of toxic substances in the heartwood (Panshin & de Zeeuw 1980). Decay or rots refer to the characteristic softening, discoloration and eventual disintegration of wood by fungi. They are the major type of damage and problem in the growth of wood caused by fungi. Wood that is already affected is known as decomposed or decayed wood. According to Haygreen & Bowyer (1996), decay fungi cause significant softening and or weakening of wood often to the point that its physical characteristics are completely destroyed. Decay also makes changes to wood in their appearance, physical and chemical properties (Zabel & Morrel 1992).

There are four elementary conditions to be met in order for fungus to grow which is include temperature rate, adequate supply of oxygen, sufficient of moisture and also food supply. The presence of decay in wood is also dependent on the growth rate of fungi (Panshin & de Zeeuw 1980). Staining and decay will grow over a temperature range of 0°C until 45°C and for

moisture contents, in the range 40% until 80% is optimum for fungal growth (Zabel & Morrel 1992). These levels of moisture guarantees sufficient free water accelerate the chemical reactions taking place for the period of degradation. However, most decay fungi are mesophilic and grow at temperature in the range between 10°C to 40°C with optima at 20°C to 30°C (Eaton & Hale 1993). Besides, moisture content 20% of more on wood is attained it become susceptible attack by fungi otherwise some of fungi differ in their ability to tolerate low moisture conditions (Eaton & Hale 1993).

**1.2 Soft rot decay**

Soft rot is the structure of fungal decay caused by ascomycetes and fungi imperfecti / deutromycetes (Hong & Wong 1993). These organisms cause a gradual progressive degradation from the outer surface of wood inward (Haygreen & Bowyer 1996) and they are preferentially attack cell-wall carbohydrate or eroding the wood cell walls (Zabel & Morell 1992; Eaton & Hale 1993). The appearance of soft rotted wood are dark, brown and rotted wood break easily with a brash hairline fracture. Soft rot fungi occur mainly under conditions where the growth and activities of the generally more actives and competitive basidiomycetes decay fungi are retarded (Eaton & Hale 1993). The soft rot fungi are found growing on wood with high moisture content and their activity become greater in nutrient-rich soil (Eaton & Hale 1993). Besides, factors including high temperatures and concentration of soluble nitrogen may also favor sof rot (Eaton & Hale 1993). Soft rots fungi are an example of decay that are capable of attacking wood under subtropical and tropical environment. Meanwhile, the soft rot wood decay is reasonably similar to brown rot as far as lignin is slowly being degraded (Hong & Wong, 1993) or in other cases, the soft rot fungi cannot degraded lignin totally (Panshin & de Zeeuw 1980 ). Soft rot fungi most often attack wood that is very wet

and typically penetrate wood rather slowly (Panshin & de Zeeuw 1980; Wong 1988; Eaton & Hale 1993; Haygreen & Bowyer 1996). A soft rot fungi, *Chaetomium globosum* fungus also has been used as a standard fungus for soft rot decays tests for many years. As reported by Hong & Wong (1993), recent studies at FRIM propose that copper- chrome-arsenic (CCA) may less efficient against soft rot decay.

**1.3 White rot decay**

White rot fungi are basidiomycetes which causes substantial and breakdown of lignin in wood (Eaton & Hale 1993; Panshin & de Zeeuw 1980; Haygreen & Bowyer 1996; Hong & Wong 1993). These fungi typically erode outward from the cell lumen by decomposing consecutive layers of the cell wall much as a river erodes its bank (Haygreen & Bowyer 1996). White rots may change the colour on wood slightly but more often give it a bleached or whitish colour (Hygreen & Bowyer 1996) as tannins and extractives are removed and lead the reduced fibrous mass (Rossmore 1995). Cellulose and hemicelluloses are also degraded substantially but at varying proportions depending on the fungal species. In a number of white rot fungi, the type of attack may restricted by nutritional factor. Comparing brown rot fungi, white rot fungi also involves degradation of all major structural chemical component of the wood but brown rot involve removal of carbohydrates components only (Rayner & Boddy 1988). According to Haygreen & Bowyer (1996), there is great variability in the mode of action of white rot fungi and mycologist and forest product pathologists are attempting to better understand the mechanism through which these fungi degrade wood substances.

### 1.4 Brown rot decay

This form of fungal decay is also caused by basidiomycetes that selectively attack the cellulose and hemicellulose of the cell, with slightly effect on the lignin (Haygreen & Bowyer 1996 ; Hong & Wong 1993). Wood seriously degraded by these fungi will have an abnormally brownish or reddish color. These fungi may attack all layers of the cell wall, but the cellulose in the S-2 layer is often the first to be degraded (Haygreen & Bowyer 1996). In the final stages of brown rot, the decayed wood is converted into a dusty mass of varying shades of brown (Panshin & de Zeeuw 1980). The strength of timber that already attacked by brown rot becomes seriously affected through a loss of hardiness and an increase in brittleness (Rossmore 1995).

### 1.5 The need for most Decay susceptible wood as control in Decay Test.

In lab studies, it is shown that over a prescribed duration of testing (up to 3 months), it is desirable to have a decay susceptible wood control which can yield high mass loss up to 60% according to the decay test method of American Standard Testing and Materials (ASTM). Often, the mass loss achieved by some decay susceptible timbers, however, failed to reach 60% after a fixed decay period. It is of interest to find a decay susceptible timber that yields such high mass loss in the shortest decay period.

## 1.6 OBJECTIVES

1) To determine, from selection of non-durable Malaysian timbers which would be most susceptible (lowest durability to decay) and can thus serve as a control specimen in decay tests.
2) Compare the relative rates of decay between the non-durable woods over several weeks of exposure.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Decay Susceptible Timbers

Timbers that only contain sapwood and non-durable heartwood are obviously decay susceptible. Some among these timbers are probably more decay susceptible than others over a fixed decay test period assesed for six weeks or up to three month. According to Wong (1988), Rubberwood and Kempas are respectively the common light hardwood and medium hardwood that are being attacked by soft rot, brown rot and white rot fungi. Besides, Rubberword is categorized as non-durable and most susceptible wood compared with other light hardwoods ( Wong & Sabri 2000). Rubberwood and similar types of light hardwoods such as Jelutong and Ramin are awfully susceptible to blue-staining predominantly (Wong & Singh 1998) and compared to other non-durable hardwoods such as Punggai (*Coelostegia griffithi*), Gaham badak (*Bluemeodendron tokbrai*), Jelutong (*Dyera costulata*), Ludai (*Sapium* sp) and kayu arang *(Diospyrus* sp), Rubberwood appears to be most susceptible to soft rot decay that caused by variety of microfungi, as reviewed Hong & Wong (1994) and studied by Wong (1993).

The presence of reserve foods in the parenchyma cells of their sapwood may increase its susceptibility to decay particularly to bacterial and fungal staining (Wong & Singh 1998). This is because the reserve food will attract fungal organisms. However, even the sapwood of all native species in which the heartwood is highly durable, is non susceptible to deterioration by biological agents because it lacks extractives in sufficient quantity or toxicity to reduce the

growth of microorganism (Panshin & de Zeeuw 1980). Besides, factors other than extractives which are expected to determine variation in susceptibility timbers are chemical and anatomical in nature (Wong *et al.*, 1983; Eaton & Hale 1993). This is because the shape and dimensions of cells and the abundance and structure of pits may influence microbial colonization of wood. For the chemical structure, these include the content of non-structural carbohydrates, nitrogen and a variety of minerals, proportions and nature of structural cellulose, hemicelluloses and lignin.

### 2.2 Decay Test or Durability Test Method

According to Roosmore (1995), decay test methods are often used to evaluate the decay resistance of timbers as well as evaluating the preservative performance of treated wood blocks. There are two type of test method that are always been used for decay fungi attack. This method includes Petri plate test and also soil block test (Zabel & Morrel 1992).

#### 2.2.1 Petri plates test

This test method is suitable for studying decay resistance / susceptibility of wood species. It involves exposing wood blocks to a medium containing different decay fungi. Petri plates test is the European standard EN 113 and based on malt extract agar which can be used as standard medium. The agar is introduced into round or square jar or even Petri dish and test fungus is inoculated and allowed to grow over the agar surface. An agar block method develop for soft rot provides adequate moisture, plastic mesh used as blocks support to allows moisture to equilibrate at below saturation levels (Anagnost & Smith 1997). This method provides a relative measure of the growth of fungi on artificial media which is markedly different from growth in wood. The presence of decay primarily is determined by

weight loss for the blocks and surface degrade or strength loss for the stakes (Roosmore 1995). However, factors that influence decay rate, such as moisture content, nutrient availability and accompanying fungal decay capability can be varied by using different decay chamber methods (Anagnost & Smith 1997).

### 2.2.2 Soil block test

This method is listed as Standard ASTM D 1413-76 of the American Society for Testing and Materials. In this method, a wood block is exposed to fungi in duration per decay inside the decay chamber containing soil and filter paper soaked with malt extract solution as fungal nutrient. As an application of the soil block method, wood is also treated with test chemicals at given concentrations to produce desired target retention (Zabel & Morrel 1992). After weighing to determine the amount of chemical absorbed, the blocks are dried and reweighed then exposed to one on the selected fungi. The blocks are exposed to the test fungus for a few weeks and up to three months and then removed from the bottles, oven dried and reweighed. This weight is compare to the initial treated dry weight to determine the weight loss due to fungal exposure. Soil block tests provide a relative measure of preservatives performance against white and brown rot fungi, but do not test resistance to soft rot organisms, which are known to be less sensitive to wood preservatives. This test also provides a relative guide to decay resistance, but this method cannot adequately correspond to the associations between various fungi in a natural environment.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 Woods

Wood materials were obtained from Associate Professor Dr. Andrew Wong from collections in local sawmills. Each wood was labeled as below for identification.

**Table 1:** Selected light hardwood used in this study.

| Wood materials | Labeling name |
|---|---|
| Rubberwood (*Hevea brasiliensis*) | RW |
| Kempas *(Koompassia malaccensis)* | KS |
| Seladah *(fam. Burseracae)* | SL |
| Pelai *(Alstonia* sp*)* | PL |
| Ramin *(Gonystylus* sp*)* | RM |

Wood stakes of Rubberwood, Pelai, Seladah, Ramin and Kempas heartwood species were cut into separate test blocks with measurement 2cm x 2cm x 2cm (axial x tangential x radial) containing sapwood. The entire block were weighed (oven dried weight before fungi infection in oven dry machine 105°C) and sterilized steamed two hours in autoclave machine twice a day, prior to the natural decay resistant test (Wong *et al.*, 1983).

### 3.1.2 Test Decay Fungi

Stock culture of fungi was obtained from Associate Professor Dr. Andrew Wong. The fungi were previously isolated from decayed wood. Each fungus was labeled as shown below for identification.

**Table 2:** Types of fungi used in this study.

| Fungi | Labeling name |
|---|---|
| *Chaetomium globosum* ( soft rot) | Cg |
| *Schizophyllum commune* (white rot) | Sc |

Two types of fungi, *Chaetomium globosum* (soft rot) and *Schizophyllum commune* (white rot) were inoculated on malt agar plates from the stock culture.The growth of fungi were observed for one or two weeks to see if there is no contamination. The fungi were allowed to grow until the whole surface area of malt extract agar in 8.5 cm Petri dish was fully covered with mycelia, they were reinoculate again to get the pure cultures. These pure cultures of fungi were used inside the decay chamber during the decay test.

### 3.1.3 Equipments and Apparatus

In this study ALP Model AG-23 autoclaves (121°C) were used to sterilize all the apparatus which is included the wood, soil and decay chamber before starting the decay test. This procedure is necessary to achieve better sterilization technique to avoid all the contaminant from occurring. Besides, SL SHELL LAB 1350 FX oven was used in order to oven dry wood blocks. The measurement of the oven dry weight and mycological media were made using the electronic balance. Sectioning wood blocks to prepare slides for microscopic observations of

decay patterns was made using LEICA model SM2000R microtome. Macroscopic view of wood in decay wood was captured using the LEICA camera attached to microscope.

### 3.2 Laboratory methods

#### 3.2.1 Soil preparation for decay test

Garden soil was purchased for a nearby nursery. The soil was air dried for four days. The soil analysis has to be taken which included the setting of soil moisture to 130% water holding capacity (WHC). Moisture content of air dry soil, WHC and mass of 200 ml air dry soil will measured. In determining the WHC, the soil have to be fill in buchner funnel. Then, set up contents suspended on the beaker of water and water will saturate the soil. Leave overnight to allow water to soak the soil gradually and lastly, apply the vacuum suction to the soil for 15 minutes. Then determine the moisture content of the soil as soil WHC. Formula and data for calculation the water holding capacity is showed below;

**Table 3:** Data for calculation of soil water holding capacity.

| Parameters | Measurements |
|---|---|
| Moisture content of air dry soil | 17.07% |
| Moisture content of soil (Water holding capacity) | 32.28% |
| Mass of 200 ml air dry soil | 207.03% |

$$\frac{(1.30 \times WHC\text{-}B) \times D}{100 + B}$$

WHC =Water holding capacity

B = Moisture content of air dry soil

D = Mass of 200ml air dry soil.

Through the calculation that has been done, 50ml distill water would have to be added to the soil inside the decay chamber, to bring the soil moisture content at 130 % of WHC.

### 3.2.2 Decay Test

In this study, forty test blocks for each wood species were used and subjected to decay per decay test duration (3, 6, 9 and 12 weeks), by *Chaetomium globosum* (soft rot) and *Schizophyllum commune* (white rot). Decay chambers contain 200g soil and added with 50ml distill water were prepare. The amount of distill water to add was determined by the formula shown before. After that filter paper that was already soaked with the malt extract without agar put on the top of the soil and followed by nelton mesh. The plastic tray decay chamber was autoclaved. After that, fungal inoculum was added to the decay chambers in the laminar flow and then incubated for 1-2 weeks. Then, the woods blocks were added and subjected to decay in decay chambers. One decay chamber contain 5 replicates of wood blocks which means that on single wood species provide 40 wood blocks as 20 replicates for *Chaetomium globosum* test and 20 replicates for *Schizophyllum commune* test in two separate chambers. The entire decay chambers were placed inside a large polyethylene bag covered with corrugated cardboard box sited on a bench at room temperatures (26°C-27°C). During incubation for every 3, 6, 9 and 12 weeks, 5 replicates blocks / fungus / wood species were removed from the chamber and then oven dried mass loss of these wood blocks were compared to the oven dry mass before infection to calculate percentage mass loss.

### 3.2.3 Mass loss and final moisture content of wood

The mass loss of wood blocks is the main decay parameter used, to compare decay between wood species. Therefore, oven dried mass of wood before infection and oven dried mass after infection were conducted appropriately. Oven dried mass of wood before infection were carried out before the decay test while oven dried mass after decay test. The formula for mass loss is;

$$\text{Mass loss} = \frac{WA - WC}{WA}$$

WA= Oven dried weight before infection

WC= Oven dried weight after infection

The moisture content of wood after decay was determine in order to observed the interaction between mass loss and moisture content. The formula for FMC of wood blocks were shown below;

$$\text{Moisture content} = \frac{WB - WC}{WC}$$

WB=Fresh mesh after infection

WC= Oven dried weight after infection

### 3.2.4 Light Microscopy

The woods were cut using microtome to make a slice with size 18-20μm thick. After slice of wood was made, it was transferred into safranin and transferred continuously into ethanol with different concentration and lastly into xylene. The thin sections were placed on the slide and added with Canada balsam before covered with slide cover. Microscopic images were captured to observe the pattern of fungal attacked among five susceptible wood species.

### 3.2.5 Statistical analysis

Data for all observations were analyzed using three-way ANOVA and T statistics (multiple comparisons of means) applied using Least Significance Difference at 5% probability. The data was including the levels of decay between test period, types of fungi, and wood species. Data will be analyzed using Microsoft Excel and MINITAB version 13 softwares. The calculated LSD values used this formula;

$$LSD = t_v\,(0.025) \times \sqrt{\frac{2 \times MSE}{N}}$$

MSE = Mean Standard Error

N = Cell replication

$T_v$ (0.025) = Based on error degree of freedom.

# CHAPTER FOUR

# RESULTS

## 4.1 Decay test

Table 4 below shows the results for three-way analysis of variance for mass loss. There were significant difference ($P < 0.05$) of wood mass loss in terms of relationship between weekly interval of observation ($F= 100.63$), wood species ($F = 155.6$), week-fungi ($F = 6.4$), week-wood species ($F = 36.76$), fungi-wood ($F = 22.02$), and week-fungi-wood species ($F = 18.24$). Both types of fungi in combination ($F = 0.04$) did not show significant difference at level ($P < 0.05$).

**Table 4:** Analysis of variance for percentage of mass loss.

| Source | DF | SS | MS | F | P | |
|---|---|---|---|---|---|---|
| Week | 3 | 1548.96 | 516.32 | 100.63 | 0 | s |
| Fungi | 1 | 0.22 | 0.22 | 0.04 | 0.836 | ns |
| Wood | 4 | 3193.63 | 798.41 | 155.6 | 0 | s |
| Week*Fungi | 3 | 98.54 | 32.85 | 6.4 | 0 | s |
| Week* Wood | 12 | 2263.23 | 188.6 | 36.76 | 0 | s |
| Fungi*Wood | 4 | 452.01 | 113 | 22.02 | 0 | s |
| Week*Fungi*Wood | 12 | 1123.2 | 93.6 | 18.24 | 0 | s |
| Error | 160 | 820.96 | 5.13 | | | |
| Total | 199 | 9500.75 | | | | |

F- test is significant (s) and not significant(ns) at ($P<0.05$)

New LSD for three- way ANOVA mass loss (%) = 2.8